


Fecundity assays

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 An abbreviated version of this protocol was published in eLIFE in Jan 2021

A dietary sterol trade-off determines lifespan responses to dietary restriction in *Drosophila melanogaster* females

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Detailed protocol

1. All fecundity assays were performed with the same experimental flies as for lifespans, housed under the same experimental condition (generally 10 vials, each containing 10 females).
2. after 23 hours at 25 °C the females in a vial were transferred to a new vial with fresh food.
3. The surface of the food in the vacated vials (on which the eggs were laid) were imaged using a web camera mounted on a Zeiss dissecting microscope such that the field of view captured the whole surface. When using the holidic diet, vials were backlit to increase the contrast of eggs. When using opaque food (sugar / yeast) the images were top-lit.
4. Eggs in the images were then counted either manually or using Quantifly (Waithe et al., 2015). A separate training model was generated for eggs on each of the different dietary sterol concentrations due to variance in food opacity which influences the accuracy of Quantifly.
5. Generally, eggs were counted once per week over the first three or four weeks of each lifespan. Sampling points were selected early in adult life as they cover the period of heaviest laying and are therefore indicative of relative lifetime fecundity in *Drosophila melanogaster* females (Chapman and Partridge, 1996).
 - a. For the holidic medium experiment egg counts were performed on days 4, 16, 19 and 27.
 - b. For the rapamycin experiment egg counts were performed on days 6, 8, 13, 15, 20, 27 and 34.
 - c. for the sugar yeast (SY) experiment egg counts were performed on days 6, 14 and 24.
 - d. for the yeast extract (YE) experiment egg counts were performed on days 7, 13, 20 and 28.
6. To calculate the index of lifetime fecundity:
 - a. The mean number of eggs laid per female (i.e. the vial sum divided by the number of females in the vial) was calculated for each egg count day
 - b. The number of eggs per female in each vial was summed over the days on which counts were made.
 - c. These cumulative egg lay values for each vial were the replicates used to calculate the index of lifetime fecundity across vials

References:

- Chapman, T., and Partridge, L. (1996). Female Fitness in *Drosophila melanogaster*: An Interaction between the Effect of Nutrition and of Encounter Rate with Males. *Proceedings of the Royal Society of London B: Biological Sciences* 263, 755–759.
- Waithe, D., Rennert, P., Brostow, G., and Piper, M. (2015). QuantiFly: robust trainable software for automated *Drosophila* eggcounting. *PLOS One* 10.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Zanco, B. and Piper, M. (2021). Fecundity assays. *Bio-protocol Preprint*. bio-protocol.org/prep1114.
2. Zanco, B., Mirth, C. K., Sgrò, C. M. and Piper, M. D. (2021). A dietary sterol trade-off determines lifespan responses to dietary restriction in *Drosophila melanogaster* females. *eLIFE*. DOI: [10.7554/eLife.62335](https://doi.org/10.7554/eLife.62335)

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